J. Dairy Sci. 99:1–13
http://dx.doi.org/10.3168/jds.2015-10438

Analysis of production responses to changing crude protein levels in lactating dairy cow diets when evaluated in continuous or change-over experimental designs[1]

G. I. Zanton[2]
USDA-Agricultural Research Service, US Dairy Forage Research Center, Madison, WI 53706

ABSTRACT

The objective of this study was to evaluate by meta-analysis the effect of experimental design on the production response functions obtained when changing crude protein (CP) levels in lactating dairy cow diets. The final database of studies meeting the selection criteria contained 55 publications with 23 classified as using a continuous (80 diets) and 34 classified as using a change-over (173 diets) experimental design (2 publications reported results from trials using both designs). Mixed model, weighted analysis of covariance was conducted on production measures in response to CP including the continuous covariates year of publication and average days in milk (DIM) and the discrete classification covariate of experimental design. The model was evaluated for curvilinearity in the response to CP, variance heterogeneity, and coincidence in the response between experimental designs, with $P < 0.05$ indicating significant effects and $P < 0.15$ indicating trends. On average, cows in experiments using continuous designs had a lower initial DIM, were on treatment longer, and produced a greater yield of milk and milk protein than cows in experiments using change-over designs. Production responses to increasing CP were increased dry matter intake (DMI), milk and component yield, and feed efficiency (milk Mcal/kg of DMI), and decreased milk N:intake N. Response in milk yield and feed efficiency to increased CP interacted with experimental design where continuous experiments had greater milk yield and feed efficiency response at higher levels of CP. Interaction between CP and design effects on yield of milk protein and fat or milk N:intake N did not approach significance. The database is limited by the lower number of continuous studies and the differences in average DIM between designs; nevertheless, it is concluded that DMI, milk protein yield, milk fat yield, and milk N:intake N responses to CP did not depend on experimental design. Response of milk yield and feed efficiency to CP interacted with experimental design; however, prospective research on the influence of experimental design is required to test these results.
Key words: crude protein, milk production, experimental design

INTRODUCTION

When designing experiments to test different dietary treatments fed to dairy cows, the researcher is required to balance many design parameters that could potentially affect the inferences made after the experiment has been concluded. Many decisions at this stage of experimental planning are made in an attempt to balance finite resources with obtaining sufficient statistical power to detect biologically or economically relevant treatment differences. Due to the relatively high cost of conducting feeding experiments with dairy cows and the practical significance of small treatment differences when extrapolated to a commercial scale, change-over designs are very attractive experimental designs that provide a good balance to both of these considerations. The power of these designs derive from the characteristic that treatment differences are compared within cows, because cows contribute a high amount of variance external to the treatments under examination. As a result, fewer cows are required to obtain a desired level of power than continuous experimental designs (i.e., randomized complete blocks, and so on) because treatment comparisons are made based on between-cow information in these designs.

Power calculations, replication, blocking, covariate periods, and so on are statistical devices that are used to increase the precision of an experiment (Cochran and Cox, 1957). Inaccuracy in response estimates cannot be

Received September 23, 2015.
Accepted February 18, 2016.
[1]Mention of any trademark or proprietary product in this paper does not constitute a guarantee or warranty of the product by the USDA or the Agricultural Research Service and does not imply its approval to the exclusion of other products that also may be suitable.
[2]Corresponding author: geoffrey.zanton@ars.usda.gov

corrected by means of these approaches. Using change-over designs in nutrition studies has been proposed to result in inaccuracies due to the previous nutritional condition influencing the measurements in the current period (Morris, 1999). Previous planes of nutrition are known to possibly result in significant productive effects after that plane of nutrition has been adjusted (Wilson and Osbourn, 1960; Ferrell et al., 1986). However, the extent to which these effects may persist during subsequent periods and are of a magnitude that could be classified as carry-over effects in a properly implemented change-over design is not well studied.

In one of the first publications to emphasize the implications of separating the direct from carry-over effects, Cochran et al. (1941) observed that failing to adjust for carry-over effects when different planes of feeding were provided to dairy cows resulted in an underestimation of treatment differences by ~11%. In a series of experiments, Kreuzer and Kirchgessner observed that, in some cases and for some responses, effects of previous treatments were still present after 21 d on a different diet (Kreuzer and Kirchgessner, 1985a,b; Kirchgessner and Kreuzer, 1985). Cows respond to fluctuations in nutrient intake by coordinating body tissue and milk synthesis to maintain homeostasis and homeorhesis (Bauman and Currie, 1980); this coordination appears to occur rapidly for both short-term (Bjerre-Harpøth et al., 2012; Yoder et al., 2013; Ferraretto et al., 2014) and intermediate-term (Gross et al., 2011) fluctuations in nutrient intake. It is unclear the extent to which these responses are sustainable under conditions of long-term feeding. However, timing, duration, and CP level may all be important and animals to which dietary CP level is changed may fail to respond completely under some cases of longer term differences in CP concentration (Law et al., 2009).

In a comprehensive statistical analysis, Huhtanen and Hetta (2012) concluded that change-over designs were as accurate as continuous studies when expected treatment differences were not extreme. On average, the studies entering that analysis were conducted in 1988–1993 with 17.2 to 18.3 kg of DMI and 24.5 to 25.9 kg of milk yield and thus represent a different population of cows and nutrient demand than would be generally applicable for current and future research. The feeding protocols for the studies that entered the analysis by Huhtanen and Hetta were stated to primarily have been based on allocating a fixed amount of concentrates and allowing ad libitum consumption of forages, typically grass silage. A frequent response observed when CP% is increased is an increase in DMI (Rico-Gomez and Faverdin, 2001; Huhtanen and Hristov, 2009); given the fixed rate of concentrate feeding in the previous analysis, it is questionable whether the responses would be similar when forage and concentrates are both offered for ad libitum consumption in a fixed proportion as is the case with a TMR. In addition, the response functions presented (Huhtanen and Hetta, 2012) were linear in nature and without accounting for potential diminishing or negative returns as has often been shown in nutrition. In consideration of these questions and to obtain a better understanding of the implications that choice of experimental design has on the inferences on protein nutrition, the literature was analyzed through a meta-analysis with the objective of evaluating the effect of experimental design on the response functions obtained when feeding different levels of CP.

MATERIALS AND METHODS

To accomplish this objective, a database of studies that had the principal objective of evaluating the effects of changing the level of CP on the productive responses in lactating dairy cows was established. The following criteria were used to allow inclusion in the database: (1) published since 1990 in the *Journal of Dairy Science*, (2) conducted in North America with the predominant forage sources coming from corn silage or alfalfa, (3) the experimental objective of the trial was to evaluate different levels of CP in early- to mid-lactation ($0 <$ starting DIM < 200), (4) experimental design was described (including the number of animals per treatment or total, DIM, period length, adaptation period length), and (5) diet CP content, DMI, milk yield, and milk components were reported. Date range, journal, trial location, and forage source inclusion criteria were used in an attempt to reduce the heterogeneity within the population of studies that entered into the analysis. Experimental designs were classified as continuous when treatment diets were fed to cows for the entire treatment period (not considering the dietary change that occurs at the initiation of the study) or change-over when 2 or more dietary CP concentrations were fed over the course of the study.

The scope of the meta-analysis was purposely restricted to where CP levels changed so as to have an independent variable that is essentially always determined from chemical analyses and reported in the publication, therefore not dependent on book values or model predictions (as opposed to, for example, MP, which could be predicted to change due to altering rumen degradability or due to incidental changes through changes in energy formulation or DMI) and because of an hypothesized sensitivity in productive and adaptive responses to CP alterations. Experiments were coded as trials when ≥ 2 CP levels were fed in a study. Several trials included treatments with 2 levels